GEL PERMEATION FOR CLEANUP IN PESTICIDES RESIDUE ANALYSIS

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USE OF GEL PERMEATION CHROMATOGRAPHY FOR THE CLEANUP OF SAMPLES FOR THE ANALYSIS OF PESTICIDES, HERBICIDES AND OTHER TRACE ORGANIC POLLUTANTS

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ABSTRACT.

Application of Gel Permeation Chromatography as a tool for cleanup of pesticide residue samples is described. Details of the system used, are given and the effects of varying flow rate, sample concentration and lipid loading, are presented both in numerical and graphic form. Possible improvements are described, including the option of automation. Recovery data are listed for a broad range of organochlorine, organophosphate pesticides, PCB's, and phthalic acid ester plasticizers.

INTRODUCTION

Many cleanup techniques have been used to prepare dirty sample extracts for high sensitivity Electron Capture Gas Chromatography, to determine trace levels of pesticides, herbicides and other refractory organic pollutants. These techniques include adsorption chromatography, solvent partition, volatilization, and low temperature precipitation of lipids.

One principle which, until recently, has not been thoroughly investigated for this purpose is that of separation by molecular size. The technique of Gel Permeation Chromatography uses this principle. Insoluble polymer gels, with lattice holes tailored to exclude molecules above a certain size, can be used in a flow system to separate these molecules from those of smaller size. Graduated size separations are also possible below the cut-off limit. The use of this technique for the separation of a wide range of organic compounds, with molecular weights 1500 or larger, using Biobeads, or Sephadex LH20 and various solvents, has been reviewed by Mulder and Buytenhuys (1).

TABLE 1. Characteristics of the Biobeads Used.

Gel	Mol. Wt Exclusion Limit	Mol. Wt. Operating Range
Biobeads S-X1	14,000	600-14,000
Biobeads S-X2	2,700	100-2,700
Biobeads S-X3	2,000	up to 2,000
Biobeads S-X4	1,400	up to 1,400
Biobeads S-X8	1,000	up to 1,000
Biobeads S-X12	400	up to 400

Ruzicki et al (2) evaluated gel permeation using Sephadex LH20 as a means of separating various organophosphate pesticides, with ethanol as a solvent. Stalling, Tindle and Johnson have recently published papers on the use of Biobeads SX-2, SX-3 (3) for cleanup of a wide range of pesticides in oily fish extracts, prior to gas chromatography. An evaluation of their system has been published by Griffitt and Craun (4) who found it ideally suited for this type of work.

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An automated system capable of unattended processing of 24 samples by gel permeation has recently been put on the market by Analytical Biochemistry Laboratories of Columbia, Missouri, under the name GPC Autoprep 1001.

The following report describes evaluation in Ministry of the Environment Laboratories of Stalling's methodology using a simple "home-made" system.

Phase One of the project was designed to evaluate isolation of organochlorine pesticides, organophosphates, PCB's and phthalate esters from fish oil. Later phases will involve evaluation of cleanup for organophosphates, phthalic acid esters, herbicides, and other refractory organics, from matrices such as sewage extract, vegetable matter, and sediment extracts.

EXPERIMENTAL.

Table 1 lists the Biobeads products range examined, their molecular exclusion limits, and the molecular weight operating range within which separations can be achieved. Biobeads SX-2, and SX-3 were studied in these trials.

Figure 1 is a schematic of the apparatus used for this work. It consists of a solvent reservoir (A), bellows pump (B), with an air filled pulse damping loop (C), sample injection port, or valve with loop (D), followed by the gel filled column (E). In our laboratory, this was a "home-made" device constructed of 1 inch Carius tubing (Pyrex), and stainless steel Swagelok fittings. Eluates are collected in the collection bottle (F).

This system is similar to that used by Stalling. The commercial automated system is identical in principle, but includes an electronic sequencer controlling pneumatically actuated multi place wafer valves for changing sample loops and collection vessels at appropriate times. Figure 2 illustrates such an automated system in schematic form.

Solvent is pumped continuously through the system. The column is prepacked with solvent swollen gel. A bed of fine sand or a sinter can be used to retain gel beads at the exit end. Flow rates of 4-5 mls per minute are normal, higher flows causing bed compression. Sample is injected in approximately 5 mls of solvent, via the injection port (D). Solvent flow should be reduced to compensate for the increase in flow caused by the injection. Use of a sample valve and loop eliminates this problem.

For Biobeads SX-2, SX-3, lipids and materials of molecular weight greater than 600 are eluted rapidly, followed by pesticides and PCB's. For a 30 cm x 1.7 cm column of Biobeads SX-2, eluted with Cyclohexane, the elution curves for fish oil, and the DDT group of pesticides are shown in figure 3. Other chlorinated pesticides give similar curves. PCB's emerge about 20 mls later than pp'DDE.

Figure 4 (Ref.2) shows the close similarity of chromatographic traces from gel permeation and solvent partition cleanups of pesticides in fish oil. As can be seen from figure 3, not all the oil was separated. A Florisil final cleanup of the fractions was therefore necessary before gas chromatography. This double cleanup is one of the features we are trying to eliminate.

As can be seen from figure 3, although good separations of most of the lipid from pesticides was obtained, a problem was encountered with pesticide tailing. Excessively large volumes of solvent were required to recover all the pesticides (up to 500 mls). It was also noted that better separations of lipid+pesticides were obtained when equivalent lipid loadings were injected in 5 mls, than in 1 ml. Although somewhat contrary to normal chromatography practice, it was considered that this might be caused by solvation of the gel by lipids at high concentrations. This is illustrated in figure 5. (Ref.3).

Figure 6 shows the elution curves resulting from the use of ethyl acetate in place oc cyclohexane as eluant. Although some small loss in lipid/pesticide separation efficiency could be noted, the tailing problem was eliminated.

Further trials of solvent/Biobead combinations, together with consultation with Dr. D. Stalling, produced a system using a 1.7 x 50 cm column packed with Biobeads SX-3, using 25% toluene in ethyl acetate as a solvent. Figure 7 illustrates the type of separation obtained for this system. Separation efficiency was good, and no tailing was noted. The pesticide curve is an envelope of 10 common organochlorine pesticides.

Table 2 lists the recoveries and elution volumes of a wide range of pesticides. Although this work dealt basically with organochlorines, organophosphates, PCB's, and some phthalic acid esters (plasticizers) were also run. This table illustrates the broad range of possible uses this technique may have.

TABLE 2.

Compounds	Recovery %	Fraction ml
Lindane	95*	70-140
Heptachlor	96*	70-140
Aldrin	92*	70-140
Heptachlor Epoxide	99	70-140
Dieldrin	101	70-140
Endrin	97	70-140
pp'DDE	99	70-140
pp'DDD	98	65-140
pp'DDT	100	70-140
Dichlorvos	109	65-140
Phosdrin	93	65-140
Ronnel	83	65-140
Malathion	99	70-140
Parathion	92	70-140
Me. Parathion	85	65-140
Methyl Trithion	94	65-140
Ethion	89	65-150
Aroclor 1254	98	80-100
Dibutyl phthalate	103	60-100
Di-ethylhexyl phthalate	120	60-100

To more clearly elucidate the behaviour of the system, we next investigated the effects of varying the lipid loadings on separation efficiency. Figure 8 illustrates the elution curves for increasing lipid loadings. Over the range investigated, although breakthrough in absolute terms increased, percentage breakthrough varied little (less than 2%). Later experiments with a similar column indicate that above 500 mg loading, breakthrough percentage increases rapidly. Table 3 lists the amount of lipid recovered in the pesticide fraction.

TABLE 3. Fish Oil in Pesticide Fraction (70-140 ml).

Oil Injected Mg.	Oil in Pesticide Fraction Mg.
100	1.9
200	1.8
300	5.0
400	5.5
500	8.4

All samples injected in 10 ml.

The effect of varying injection volume for a fixed lipid loading was investigated next. As can be seen in figure 9, the lipid elution maxima arrive at earlier points with higher dilution, thus improving the cleanliness of the pesticide fraction. This is illustrated by table 4. Further work to determine the limits of this effect is being carried out.

TABLE 4. Effect of Injection Volume.

Sample: 100 mg Fish Oil.

Sample Volume	Oil in Pesticide Fraction (70 - 140 ml) mg
1 ml	10
2 ml	4.9
5 ml	3.5
10 ml	1.9

Figure 10 represents the chromatograms obtained by spiking a 2 gram fish sample containing approximately 200 mg fish oil with the indicated levels of DDT group pesticides. Pesticide fractions were diluted appropriately to give original spike concentrations the same as in the standard. It can be seen that at levels as low as 0.1 ppm total DDT group (individuals as low as 0.01 ppm) no problems would be encountered with determining these pesticides directly on the gel permeation pesticide fraction without further cleanup. At low concentrations (0.01 ppm) the early eluting peaks (lindane, BHC, heptachlor, aldrin) would be difficult to determine due to co-eluting lipids.

Since this work was completed, consultations with Dr. D. Stalling, and Analytical Biochemical Laboratories have indicated that by using sample injection valves and loops plus commercial, low dead volume columns and fittings, much sharper separations can be achieved, and working levels of pesticide concentration could be reduced tenfold.

Further work on such an improved system is planned, together with investigation of the possibility of coupling an automated gel permeation cleanup method with an automated gas chromatograph to cut down manpower requirements, and permit 24 hour operation.

ACKNOWLEDGEMENTS.

Many thanks to Dr. David Stalling and Mr. James Johnson of the Fish-Pesticide Laboratory, United States Bureau of Sports Fisheries, Columbia, Missouri, for their help and advice, and supply of Biobeads SX-3, at a time of dire need.

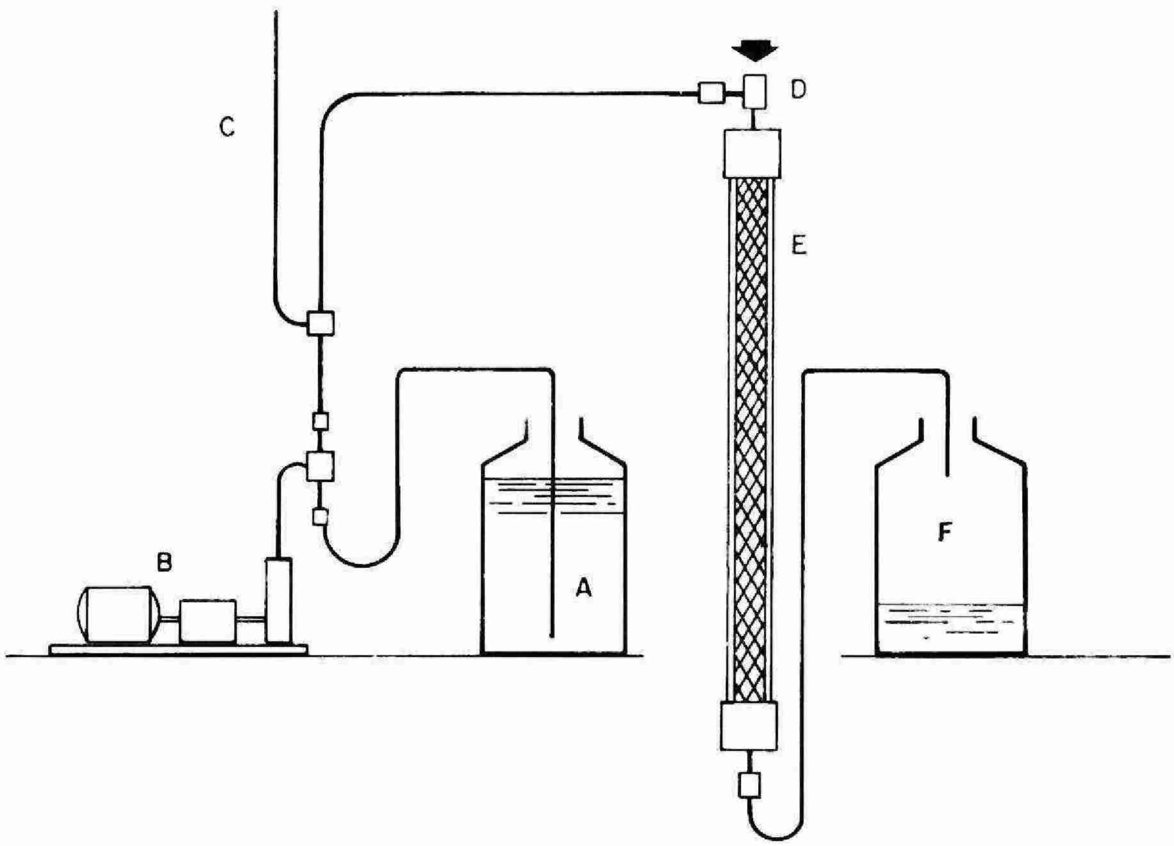

FIG. I. MANUAL GEL PERMEATION FOR LIPID-PESTICIDE CLEAN-UP
A) Solvent, B) Pump, C) Pulse dumper, D) Injection port, E) Column,
F) Collection bottle.

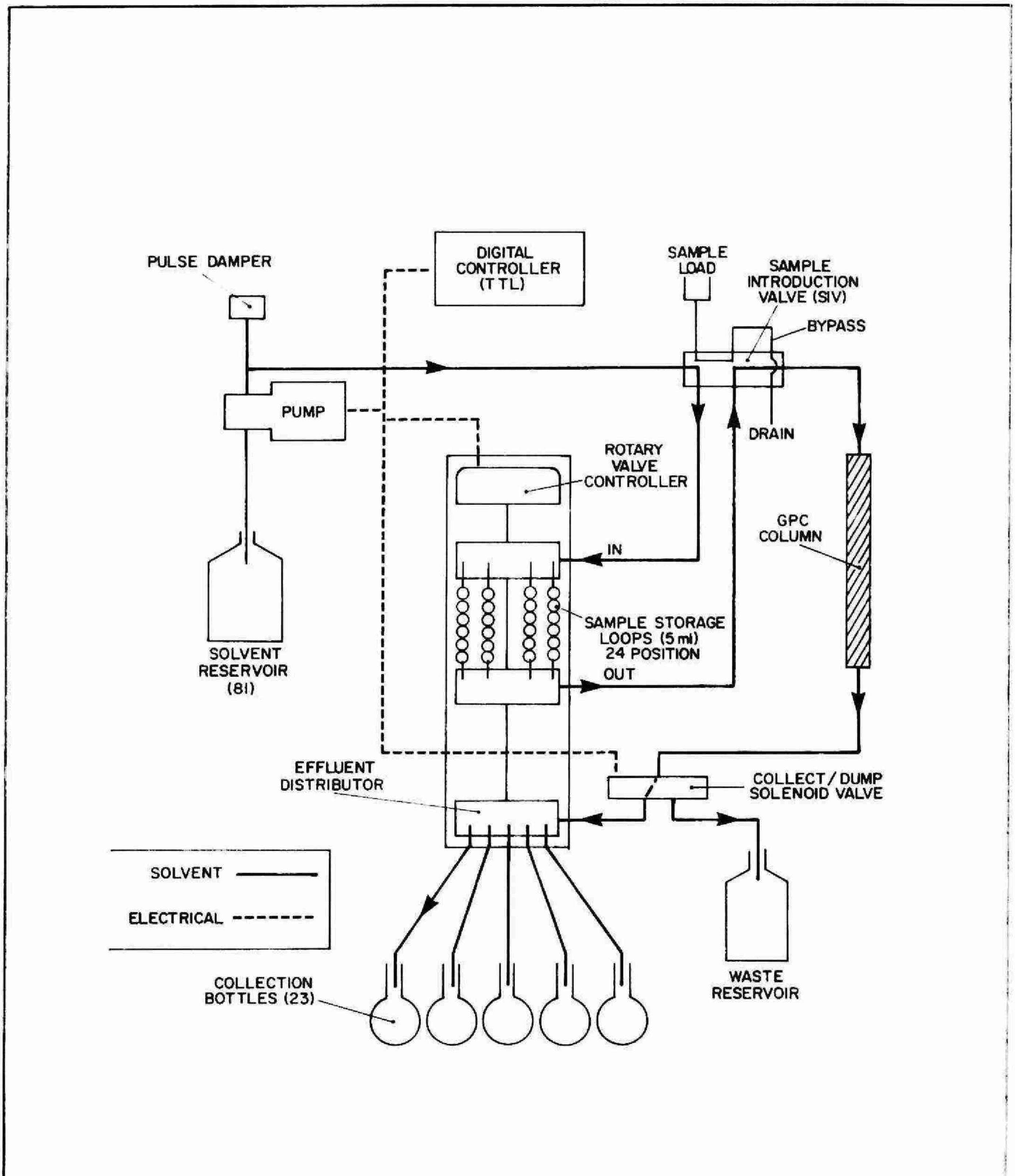

FIG. 2 GPC 1001. AUTOMATED GEL PERMEATION APPARATUS (ANALYTICAL BIOCHEMICAL LABORATORIES)

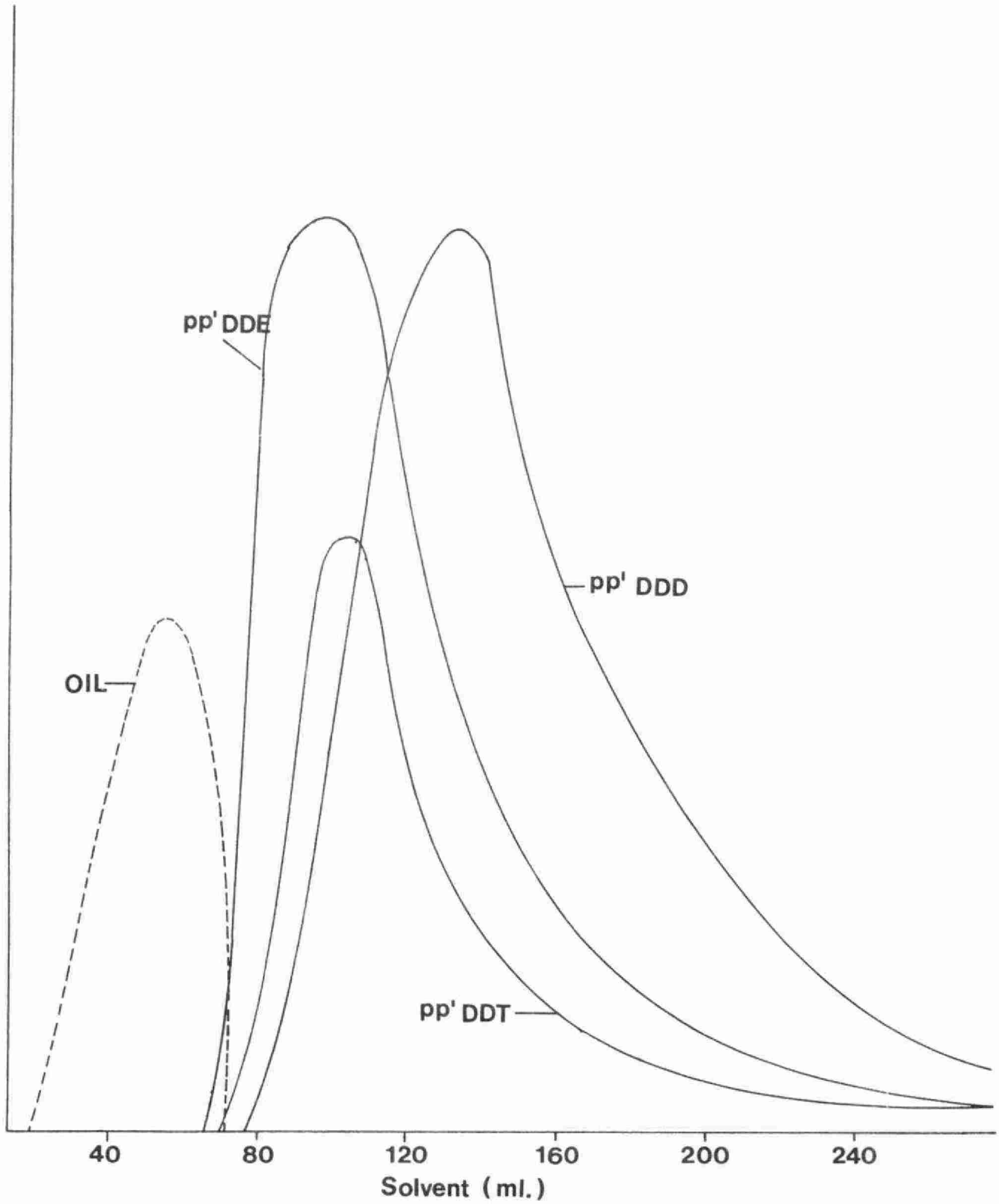

FIG. 3 ELUTION PATTERNS OF FISH OIL/DDT FAMILY PESTICIDES.
—— BIOBEADS SX-2.
COLUMN: 30 cm. x 1.7 cm., SOLVENT: CYCLOHEXANE 5 ml./min.

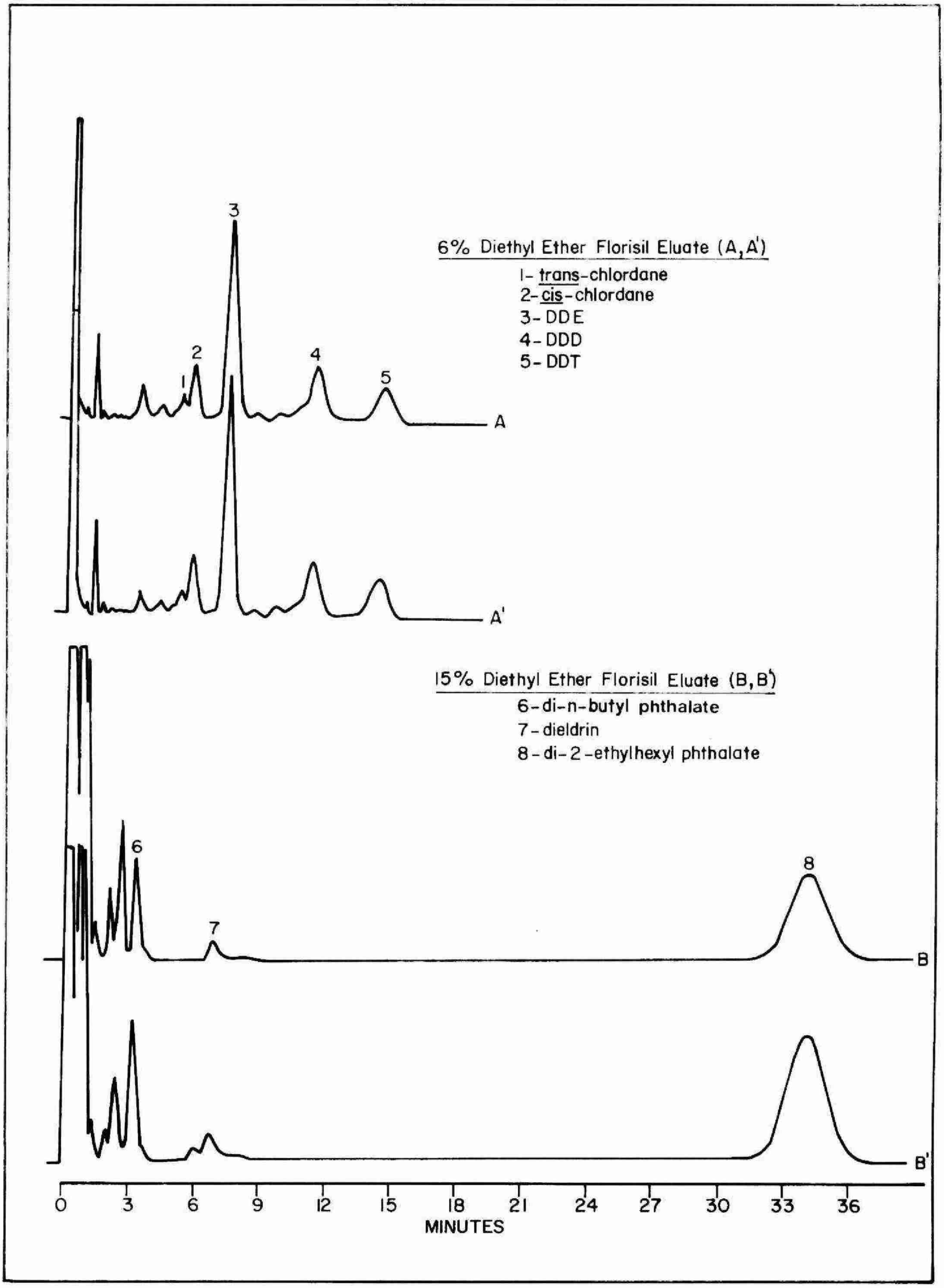

FIG. 4. COMPARISON OF ELECTRON CAPTURE CHROMATOGRAMS OBTAINED FROM A, B — SOLVENT PARTITION / FLORISIL CLEAN UP. A' B' GEL PERMEATION / FLORISIL CLEAN UP.

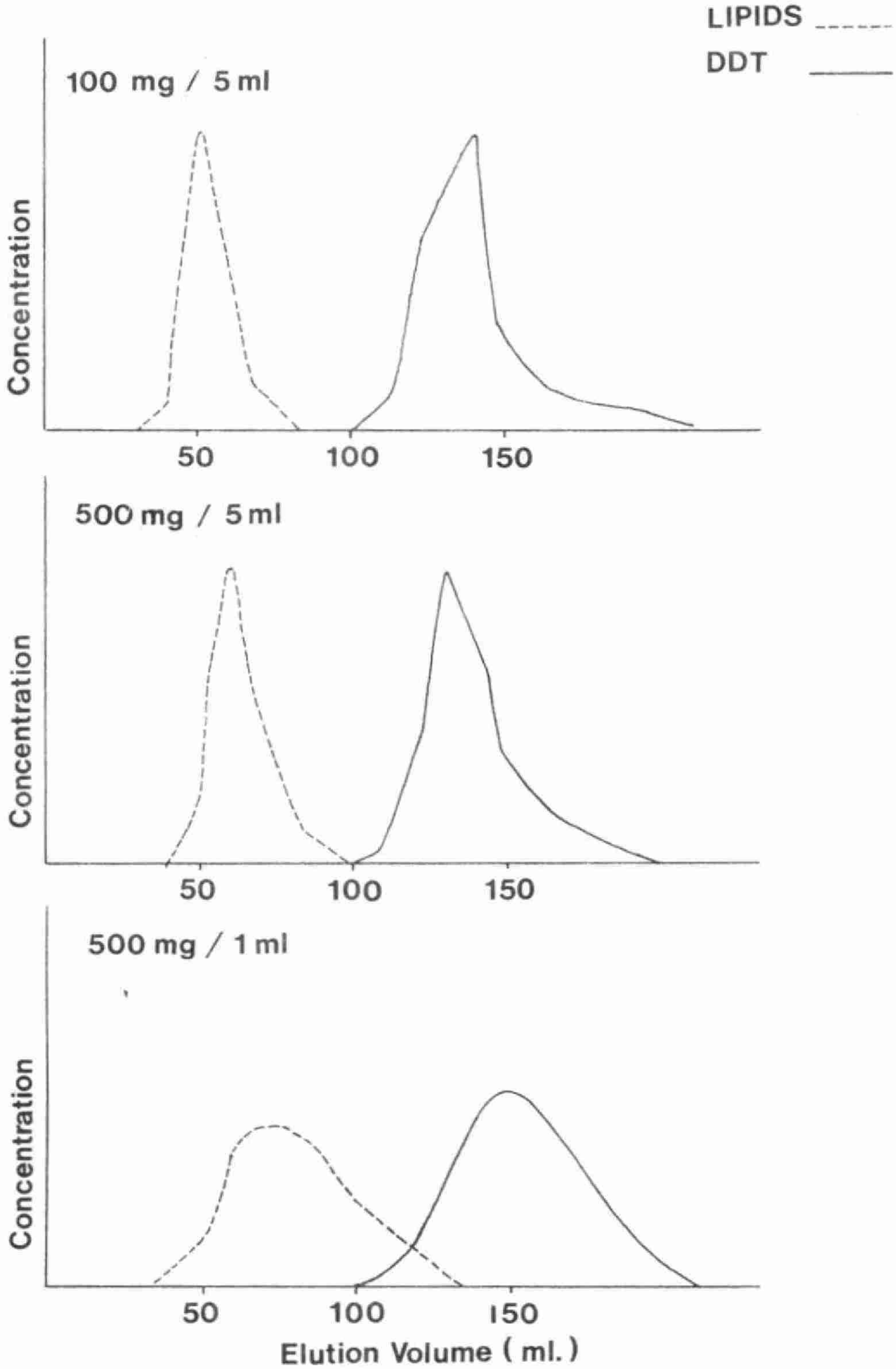

FIG. 5 GEL PERMEATION LIPID/DDT SEPARATION. EFFECTS OF SAMPLE VOLUME AND LIPID CONCENTRATION.
—— BIOBEADS SX-2.
COLUMN: 23 x 2.5 cm., SOLVENT: CYCLOHEXANE.

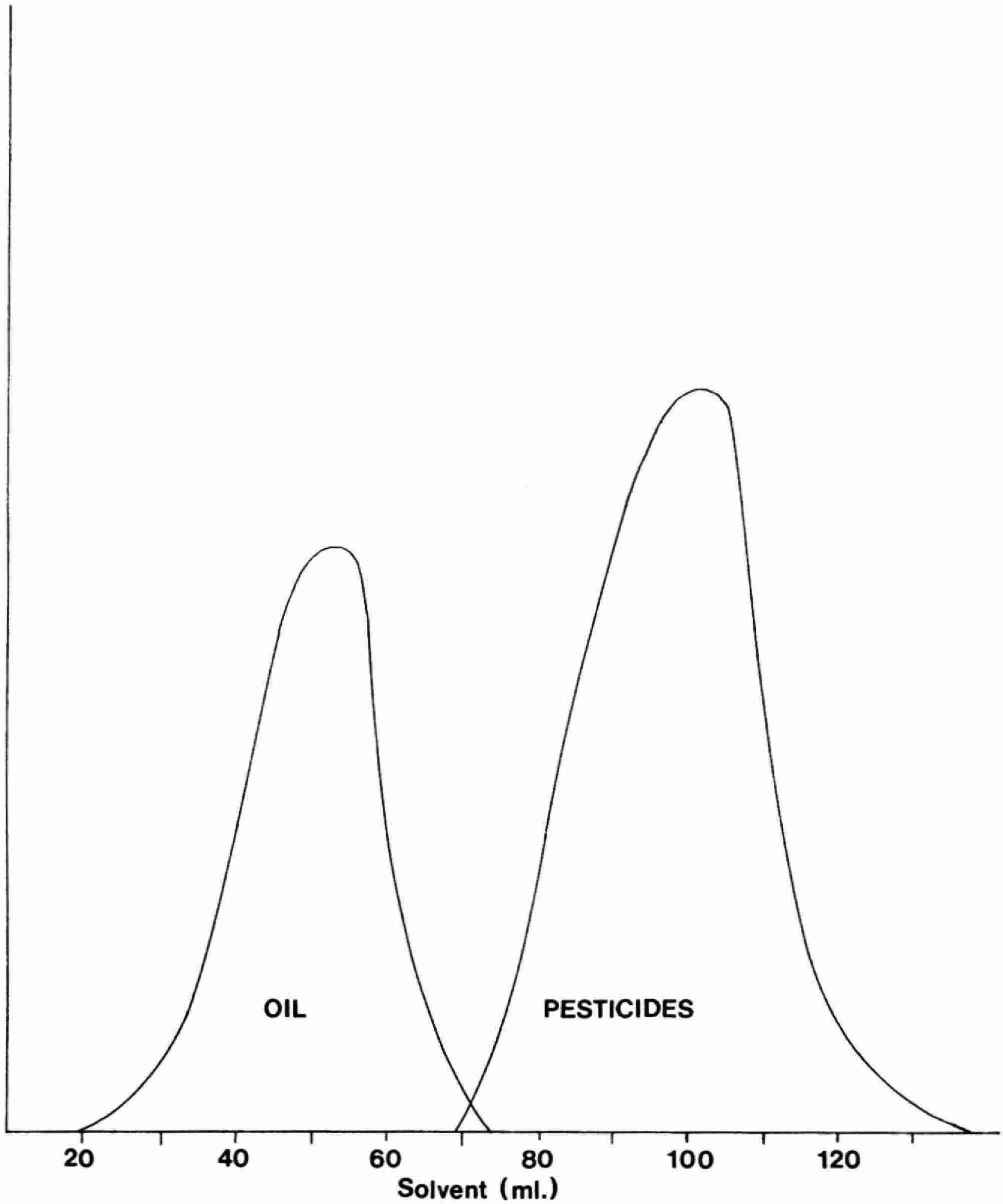

FIG.7 ELUTION PATTERNS OF FISH OIL ORGANOCHLORINE PESTICIDE (see Table 2).
——BIOBEADS SX-3
COLUMN: 50 cm.x1.7 cm., SOLVENT: 25% TOLUENE/ETHYL ACETATE.

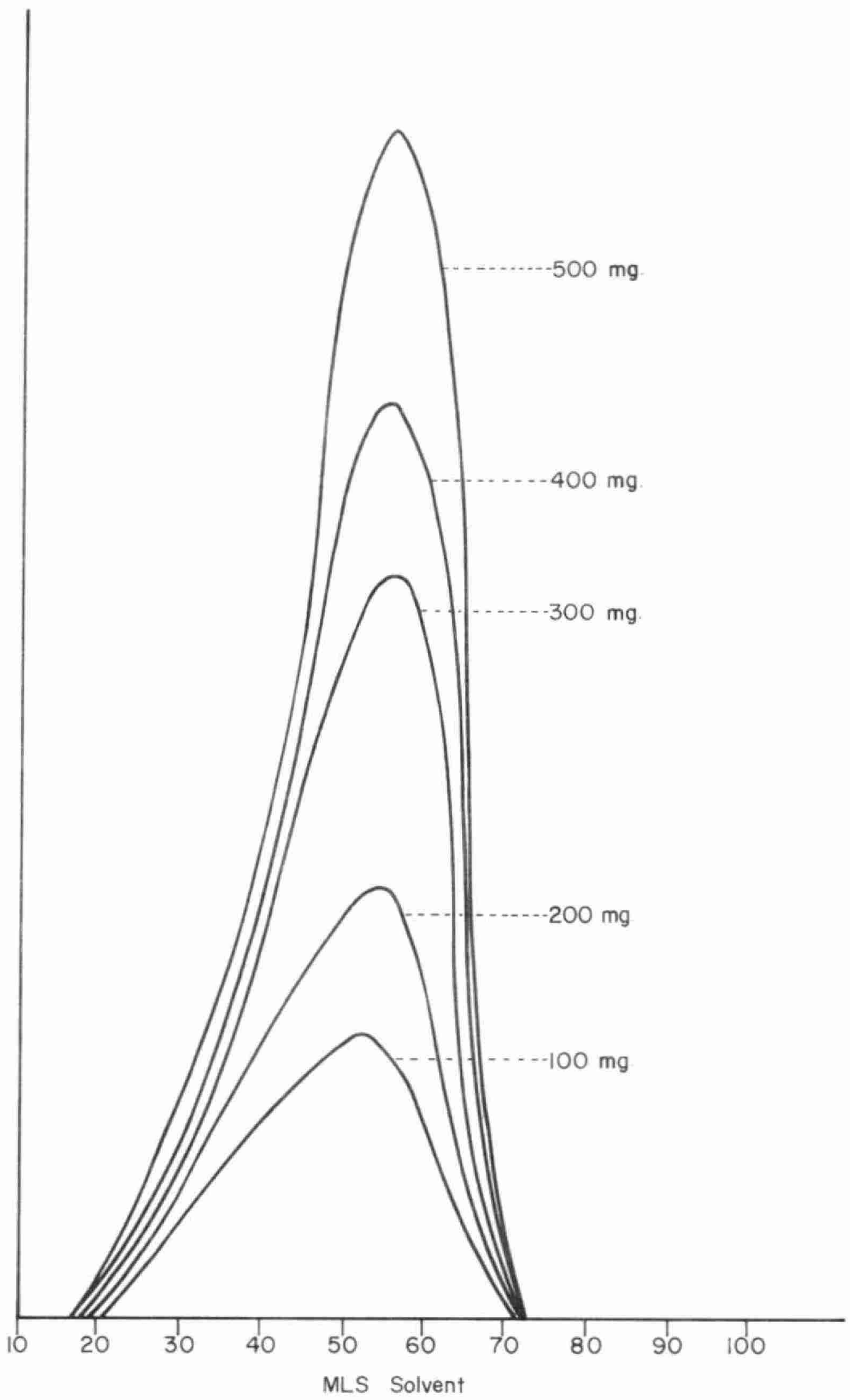

FIG. 8. ELUTION CURVES FOR FISH OIL. EFFECT OF LIPID LOADING —— BIOBEADS SX-3, USING 25% TOLUENE IN ETHYL ACETATE SOLVENT. COLUMN: 50 cm x 1.7 cm.

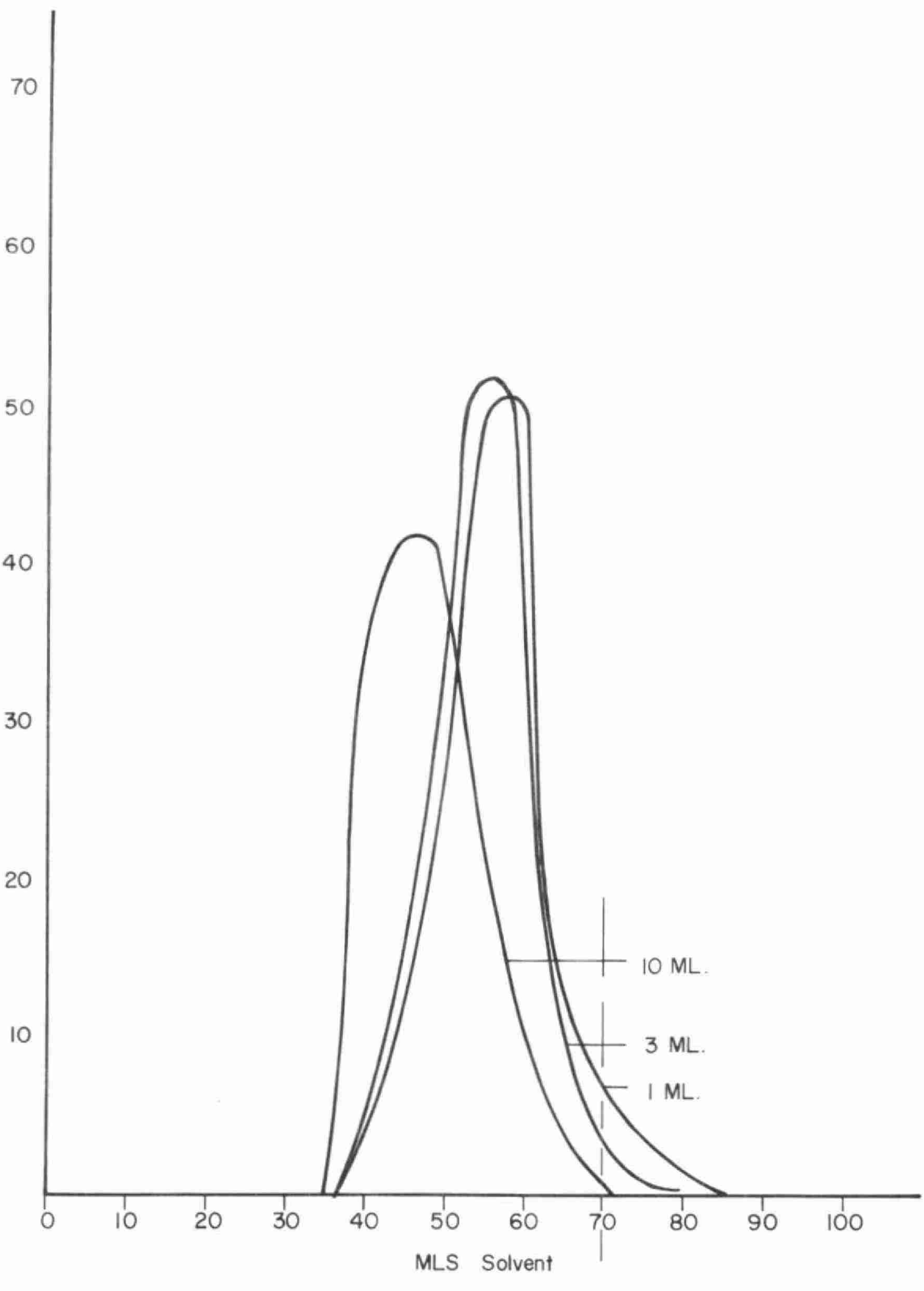

FIG. 9. ELUTION CURVES FOR FISH OIL. EFFECTS OF SAMPLE INJECTION VOLUME —— BIOBEADS SX-3, USING 25% TOLUENE IN ETHYL ACETATE SOLVENT 5 ml/min. COLUMN: 50 cm x 1.7 cm. SAMPLE: 100 mg FISH OIL.

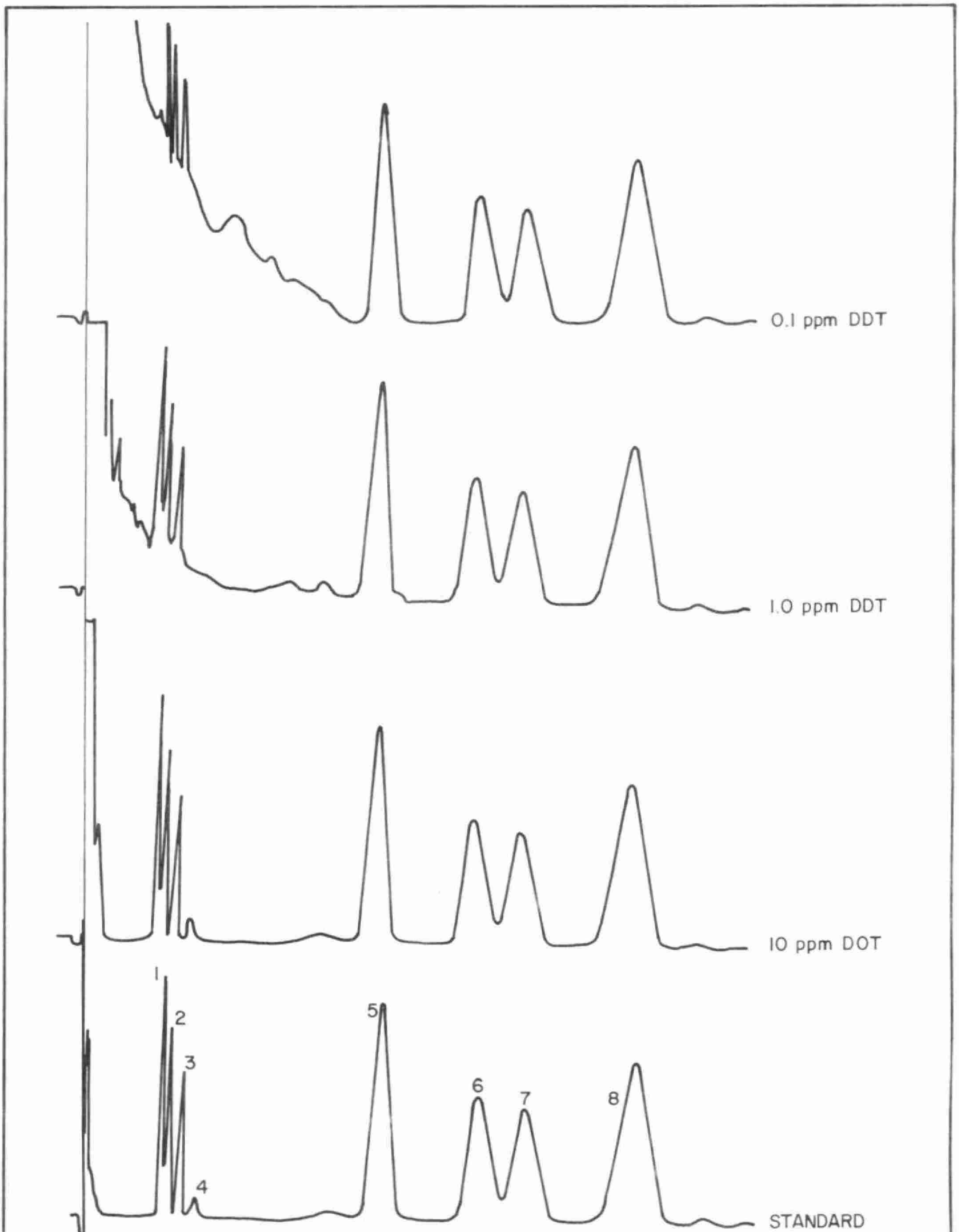

FIG. 10. CHROMATOGRAPHS OF PESTICIDE FRACTIONS FROM G.P.C. OF FISH OILS SPIKED WITH PESTICIDES AT LEVELS INDICATED. PEAKS: 1) HBC, 2) α BHC, 3) γ BHC (LINDANE), 4) β BHC, 5) pp' DDE, 6) op' DDT, 7) pp' DDD, 8) pp' DDT.